II. Remarks

A. Status of the Claims

Claims 24, 27-30 and 36-38 will be pending after entry of this amendment. Claim 31 has been cancelled herein without prejudice. Claims 24 and 36 have been amended without prejudice. Support for the amendments can be found throughout the application as originally filed, specifically, e.g., in paragraph [0366] of the Specification. Applicants respectfully submit that no new matter has been added by virtue of this amendment.

B. Claim Rejections Under 35 U.S.C. § 112

In the Office Action, claims 24, 17-31 and 36-38 were rejected under 35 U.S.C. § 112, first paragraph. Specifically, the Examiner stated that "[t]he specification, while being enabling for a method of making a polypeptide using the cell strains, substrates and growth conditions as shown in the instant specification under Example VI, does not reasonably provide enablement for a method of making a polypeptide using any cell types and under any growth conditions as now claimed." *Office Action* at pages 3-4.

This rejection is respectfully traversed. Applicants submit that the teachings of the specification, particularly under Example VI, provide enablement for a skilled artisan to make a polypeptide using any cell type under any growth conditions. However, in order to expedite prosecution of the application, the claims have been amended to limit the cell type to prokaryotic cells. Applicants submit that the specification provides enablement for a method of making a polypeptide using prokaryotic cells under the conditions of the present claims. Post filing data elucidated from experimental techniques, using the guidance of the present application and well known at the time of the invention confirms experimentally that prokaryotic cells can be used to synthesize polypeptides under the conditions of the present claims.

1. The instant application teaches how to synthesize polypeptides using prokaryotic cells

The instant application teaches that *E. coli*, a prokaryotic cell, can be used to synthesize polypeptides. *See, e.g., Specification* at ¶ [0365]-[0398]. The instant application recites that "this approach [shown in Example VI] can be applied to either prokaryotic or eukaryotic systems." *Id.* at ¶ [0366].

2. The instant application teaches how to synthesize polypeptides using both minimal growth media and rich growth medium

The instant application teaches that a polypeptide can by synthesized using both minimal media and rich growth media. "In general, cells are maintained in LB medium while expression is induced, but when it is desirable to label a protein with a radioactive isotope...M9 or MJ9 medium is used". *Id.* at ¶ [0373]. The exemplified embodiment used M9-glucose medium, a minimal medium, as the proteins were labeled for detection. Id. at ¶ [0380]. Applicants submit that the instant application clearly teaches that other growth media, i.e., rich medium such a LB, may be used.

3. The level of skill in the art enables use of any suitable growth media

Applicants submit that, in addition to the teachings of various growth media which can be used to make the polypeptides in accordance with the present invention, a skilled artisan would easily recognize a suitable selection of growth media. This position is supported by the Declaration of Dr. Kiyoyuki Matsumura at ¶ 8 (hereinafter, the "Matsumura Declaration"), attached as Exhibit A.

4. Post-filing data supports enablement for prokaryotic cells and alternate growth media

In support of Applicant's position, post-filing data is submitted herewith to show the synthesis of a polypeptide in a prokaryotic cell other than *E. coli*, and using a growth medium other than a minimal media, via the Matsumura Declaration. In the post-filing experimentation, *B. subtilis* cells were used to synthesize polypeptides using a rich medium. *Matsumura Declaration* at ¶ 9.

In order to construct the *B. subtilis* strain, tet gene, which encodes tetracycline efflux protein, was isolated from plasmid pHY300PLK and introduced into MazF expression plasmid, pMazF. The plasmid thus obtained was named pMazF-tet. *Id.* at ¶ 10. A DNA fragment containing a constitutive promoter derived from *veg* gene of *B. subtilis* was amplified by PCR from the genomic DNA of *B. subtilis*. The lacI promoter located at the upstream of *lacI* coding region in plasmid pMazF-tet was replaced with the DNA fragment amplified and plasmid pMazF-veg-tet was obtained. *Id.* at ¶ 11.

An artificial promoter having the nucleotide sequence represented by SEQ ID NO: 1, shown in Appendix A of the Mastumara Declaration, was constructed by adding the nucleotide sequence of *lac* operator and the recognition sequence of catabolite control protein A (CcpA) to *grac* promoter for an inducible expression in *B. subtilis*. The *lac* promoter in plasmid pMazF-veg-tet was replaced with this artificial promoter, and the obtained plasmid was named pN-veg-glac-c. *Id.* at ¶ 12. *B. subtilis* strain RIK1285 was transformed by plasmid pN-veg-glac-c. Several transformants were isolated after overnight cultivation on the LB agar plate containing 10 μg/ml of tetracycline. Among them, a strain of which growth was repressed on the LB agar plate containing 10 μg/ml of tetracycline and 1 mM of IPTG was chosen, and it was named *B. subtilis* strain Nvc. *Id.* at ¶ 13.

An artificial polynucleotide having the nucleotide sequence represented by SEQ ID NO: 2, as shown on Appendix A, was then synthesized. It contains the promoter sequence and the coding region for signal peptide derived from aprE gene of *B. subtilis*, multi-cloning site of plasmid pCold I, the coding region for His-Tag peptide and the transcriptional terminator sequence derived from plasmid pCold I. ACA triplet sequences in the coding region for AprE signal peptide were replaced with alternative triplet sequence without altering the encoded amino acid sequence. *Id.* at ¶ 14.

An artificial polynucleotide having the nucleotide sequence represented by SEQ ID NO: 3, as shown on Appendix A, was also synthesized. It encodes the amino acid sequence of beta-lactamase derived from *Escherichia coli* but does not contain ACA triplet sequence. *Id.* at ¶ 15.

The region spanning aprE promoter to multi-cloning site of plasmid pBE-S was replaced with the artificial polynucleotide of SEQ ID NO: 2, and then, the artificial polynucleotide of SEQ ID NO: 3 was introduced into the multi-cloning site of the obtained plasmid so that beta-lactamase having AprE signal peptide and His-Tag can be expressed under the control of aprE promoter. The obtained plasmid was named pLac-His. *Id.* at ¶ 16.

B. subtilis strain Nvc and strain RIK1285 were transformed by pLac-His, respectively. The transformants obtained were cultivated in 2 ml of KT-LB medium (LB medium containing 10 μg/ml of kanamycin and 5 μg/ml of tetracycline) at 28° overnight. 0.2 ml portion of each culture broth were added 3.8 ml of KT-LB medium and KT-LB medium containing 1 μM IPTG, respectively. The inoculated media were cultivated at 37° or 28°, and aliquots of each culture broth were took out after 5, 10 or 28 hours after the beginning of the cultivation. The proteins contained in the supernatant of the culture broth were analyzed by SDS-PAGE. The result was shown in Figure 1of Appendix B of the Matsumura Declaration. As shown in Figure 1, many

kinds of proteins as well as beta-lactamase were secreted into the culture broth of strain RIK1285 harboring pLac-His. On the contrary, the proteins other than beta-lactamase were significantly decreased in the culture broths of the transformants derived from strain Nvc as a host. In KT-LB medium containing 1 μM IPTG, the proteins other than beta-lactamase were further decreased. *Id.* at ¶ 17.

B. subtilis strain Nvc and strain RIK1285, which were both harboring pLac-His, were cultivated in 2 ml of KT-LB medium at 28° for 28 hours. The supernatants were collected from each culture broths, and the low molecular weight proteins were removed from 400 μl of each supernatant by repeating the concentration using ultrafiltration membrane and the dilution with phosphate-buffered saline, for 3 times. Finally, the supernatants were concentrated to 40 μl, and beta-lactamase activities in concentrated supernatants were assayed. The beta-lactamase activity in the culture broths of strain Nvc harboring pLac-His and strain RIK1285 harboring pLac-His were 7.2 U/μl and 0.34 U/μl, respectively. The specific activities of beta-lactamase in each culture broths calculated from these values were 61 U/mg protein and 2.4 U/mg protein, respectively. *Id.* at ¶ 18.

Based on the guidance provided by the Specification, the level of skill in the art, and the post-filing data provided in the Matsumura Declaration, Applicants submit that the present claims are enabled, specifically with respect to synthesis of a polypeptide in a prokaryotic cell under the conditions of the present claims.

Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, be removed.

III. Conclusion

If the Examiner believes that issues may be resolved by a telephone interview, the Examiner is invited to telephone the undersigned at (973)597-6162. The undersigned also may be contacted via e-mail at epietrowski@lowenstein.com. All correspondence should be directed to our address listed below.

AUTHORIZATION

The Commissioner is hereby authorized to charge any fees that may be required, or credit any overpayment, to Deposit Account No. 50-1358.

Respectfully submitted,
Lowenstein Sandler PC

Date: September 23, 2011

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EXHIBIT A